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RESEARCH ARTICLE

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Contribution to the knowledge of fungal diversity of the Marmore Waterfalls (Umbria, central Italy)

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Abstract

A list of the macrofungi collected from the Marmore Waterfalls (Umbria, Italy) is reported. In particular, a list of basidiomycetes and ascomycetes collected over a period of about ten years was compiled. A total of 125 species belonging to 78 genera, 46 families, and 15 orders were identified. Forty-four species are recorded in Umbria for the first time. Marmore Waterfalls may represent an important area for the conservation of fungal diversity, due to the presence of 34 species matching those included in Red Lists of several European countries.

Keywords

Ascomycota, Basidiomycota, ecological-trophic group, EU Habitat 7220*

Introduction

The Marmore Waterfalls (Terni, Umbria) are man-made waterfalls originally built by the Romans in 271 BC to divert the Velino River. Presently it is open to the public only when hydroelectric power is not being generated. With a total height of 165 m (541 feet), it is one of the highest man-made waterfalls in Italy and worldwide.

Due to the great biological richness in species and priority EU-Habitats, concentrated in about 0,86 km^2, it is included within the protected natural area of the 'Nera River Regional Park'. It is also recognized at the European level as a Special Area of Conservation and Special Protection Area (SAC/SPA IT5220017) of the Natura 2000 EU-wide network (Habitat Directive 92/43/EEC).

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Among the habitats, the priority EU-Habitat 7220* [Petrifying springs with tufa formation (*Cratoneurion*)] is one of the most important for its naturalistic features. Petrifying springs are lime-rich water sources which deposit tufa or travertine. The emerging spring water is rich in carbon dioxide and dissolved calcium carbonate. On contact with the atmosphere, carbon dioxide is outgassed and calcium carbonate is deposited as tufa. Communities associated with petrifying springs, namely fungi, plants and animals are highly specialized due to their challenging environment (high pH, constant inundation by water and deposition of precipitated calcium carbonate) (Aleffi and Spampinato 2009, Lyons and Kelly 2016). At sites such as waterfalls (e.g., Marmore Waterfalls) and springs, carbonate deposition is frequently extremely local (Ford and Pedley 1996).

The specific microclimate of the Marmore Waterfalls, showing high levels of ionized water aerosols, gives rise to the presence of a significant mycological diversity, which makes the uniqueness of this Site of Community Importance even more evident. A research project has been underway in this area for about ten years, leading to the census of well over 100 species of epigeal fungi.

Fungi constitute one of the largest and most significant groups of organisms in the world. They are valuable not only for their vital roles in ecosystem functions, but also for their influence on humans and human-related activities (Zervakis and Venturella 2007, Saitta et al. 2011, Pecoraro et al. 2014, Angelini et al. 2015a, 2016a, 2016b). They are responsible for a range of key ecological functions, including nutrient cycling, water uptake by plants and soil health and formation (Picco et al. 2011, Perotto et al. 2013, Frąc et al. 2018).

With regard to human-related activities, mushrooms are also involved and/or exploited in forestry, pharmaceutical industry and food production. Hence, they represent a major economic resource worldwide (Angelini et al. 2008, 2015b, 2018, Pagiotti et al. 2011, Bonanno et al. 2019). In order to maintain and to improve their strategic importance, several conservation strategies are needed (Wagensommer et al. 2018).

In this paper, a checklist of fungi occurring at the Marmore Waterfalls is reported with the aim of contributing to the knowledge about the biodiversity in this area.

Material and methods

Study area

The Marmore Waterfalls (42°33'15.56"N, 12°42'44"E), extending over an area of about 1,59 km^2 (200–360 m a.s.l.), is located in the province of Terni, the south-eastern part of Umbria (Fig. 1).

The rock wall that gives rise to the waterfall is divided into three interspersed drops. When the waterfall is closed, ponds carved into the rock by the power of water can be seen.

The study area was located on the left side of the Nera River, specifically on a series of Pleistocene terraces characterized by fluvial-lacustrine and travertine deposits (Car-

rara et al. 1993), ending at the base of the waterfall where the Velino River flows into the Nera River. The study area continues along the pathways that rise to the upper overlook. The lithology is characterized by crossings, whose deposition of calcareus travertine (or tufa) is still active, and in which there are interesting cavities. According to IT5220017 Natura 2000-Standard Data Form (http://www.regione.umbria.it/ambiente/siti-di-importanza-comunitaria-sic/), the EU-habitats investigated were: 91E0*, alluvial forests with *Alnus glutinosa* and *Fraxinus excelsior* (*Alno-Padion*, *Alnion incanae*, *Salicion albae*; 92A0, *Salix alba* and *Populus alba* galleries; 6430, hydrophilous tall herb fringe communities of the plains and the mountains to alpine levels; 7220*, petrifying springs with tufa formation (*Cratoneurion*); 9340, *Quercus ilex* and *Quercus rotundifolia* forests. They are mostly located at the base of rock faces, and subject to water spray on organic debris colonised by briophytes (Ellis et al. 2014, Poponessi et al. 2014, Ellis et al. 2016, 2017, 2018).

Macrofungal species sampling

Mycological sampling was carried out starting in 2008. Sampling was performed monthly, in particular, along the areas adjacent to the waterfall (EU-Habitat 7220*), along the different paths that go through the Park (Fig. 1).

Collections were made by samplings of each ascoma and basidioma. The surveys were limited to macromycetes that were visible to the naked eye (1 mm in size) (sensu Arnolds 1981).

The macromycetes were identified based on macro and micromorphological features according to the descriptions available in literature (Dennis 1978, Moser 1980, Jülich 1989, Candusso and Lanzoni 1990, Courtecuisse and Duhem 1994, Basso 1999, Hrouda 1999, Bernicchia 2005, Bernicchia and Gorjón 2010, Breitenbach and Kränzlin 1984, 1986, 1991, 1995, 2000, Franchi and Marchetti 2001, Robich 2003). For species names and author abbreviations, the Index Fungorum (http://www.indexfungorum.org/Names/Names.asp) and Dictionary of the Fungi (Kirk et al. 2008) were used.

The voucher specimens were dried in air-ventilated ovens at 30 °C for 72 h and deposited at the PeruMyc herbarium of the Department of Chemistry, Biology and Biotechnology of the University of Perugia (Italy).

Ecological and statistical analyses

Species richness was calculated as the number of taxa collected over the ten years. Macrofungal diversity was determined using Fisher's alpha (F), Shannon (H) and Simpson (1/D) indices (Fisher et al. 1943). Similarity of the macrofungal communities among the five EU-habitat was examined with similarity indices based on presence/absence data (Jaccard's index).

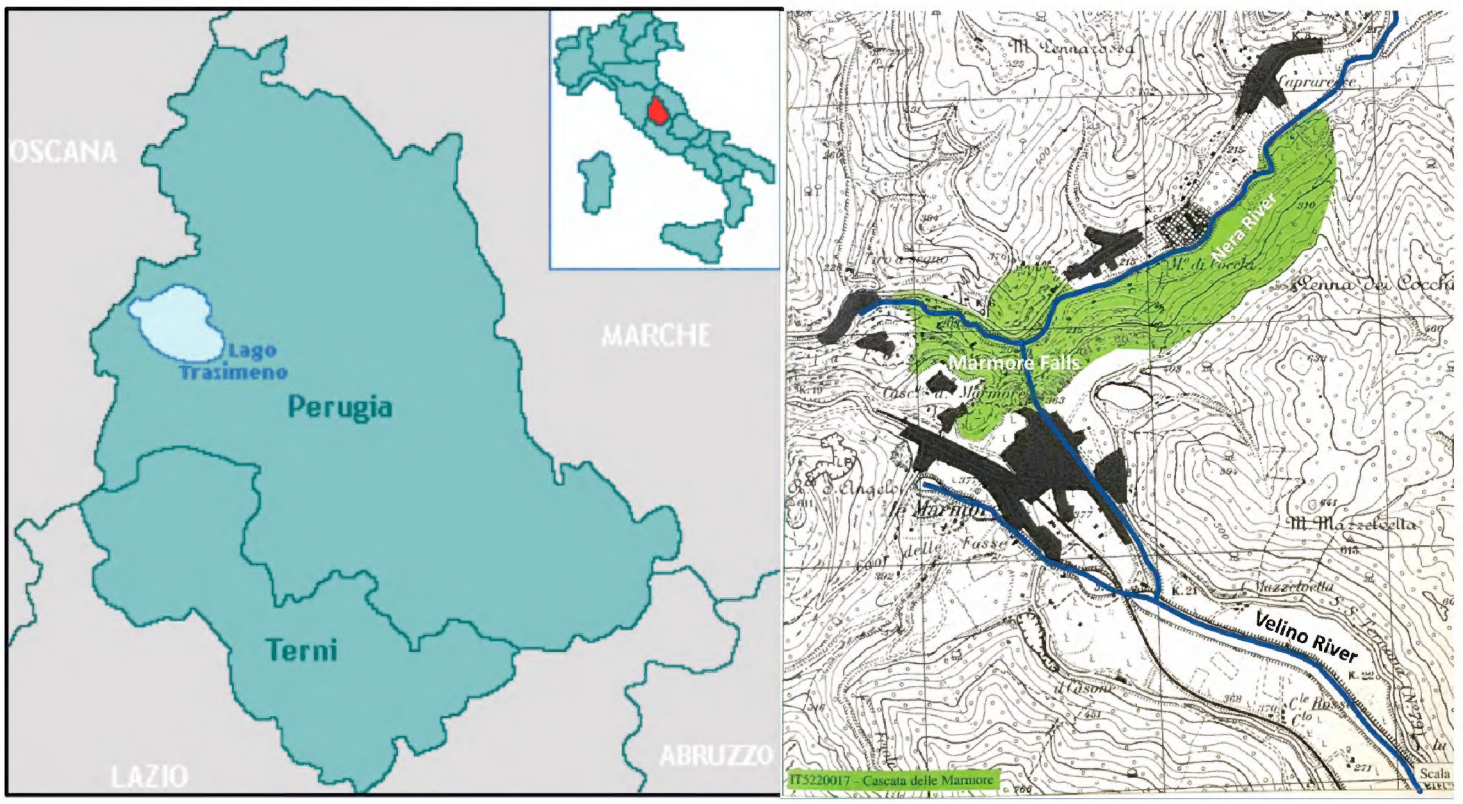

Figure 1. (draft) Marmore Waterfall Site of Community Importance according to Habitats Directive (92/43/EEC) IT IT5220017: the study area in green.

These parameters were calculated using ESTIMATES 9.1.0 (R.K. Colwell, http://purl.oclc.org/estimates). The macrofungal species were also classified into ecological trophic groups based on their primary mode of nutrition (Arnolds et al. 1995; Tedersoo and Smith 2013; Tedersoo et al. 2014).

Results

The Marmore Waterfalls, repeatedly surveyed from 2008–2018, showed the presence of 125 species belonging to 78 genera, 46 families, and 15 orders (Suppl. material 1: Table S1, Figs 2–3). Sixty-nine families belong to the Basidiomycota class and 7 to the Ascomycota.

The largest number of orders (12), genera (70) and species (107) belongs to Basidiomycota. Eighteen species, included in 8 genera, 7 families, and 3 orders belong to Ascomycota (Suppl. material 1: Table S1, Figs 2, 3). The Fisher's α, Shannon J' and Simpson (1/D) diversity indices, based on species number per genus, were 88.73, 4.18, and 52.26, respectively.

Agaricales was the most represented order, hosting the largest number of genera, followed by Polyporales and Boletales (Fig. 3). The families that had the highest number of species were Agaricaceae (10), Psathyrellaceae (9), Physalacriaceae, and Helvellaceae (7), Inocybaceae (6), and Tricholomataceae (6), which collectively accounted for approximately 30% of diversity (Suppl. material 1: Table S1). *Helvella* was the most diverse genus with 7 species, followed by *Coprinopsis* (6), *Mycena* (5), *Inocybe* (4), and *Peziza* (4) (Suppl. material 1: Table S1).

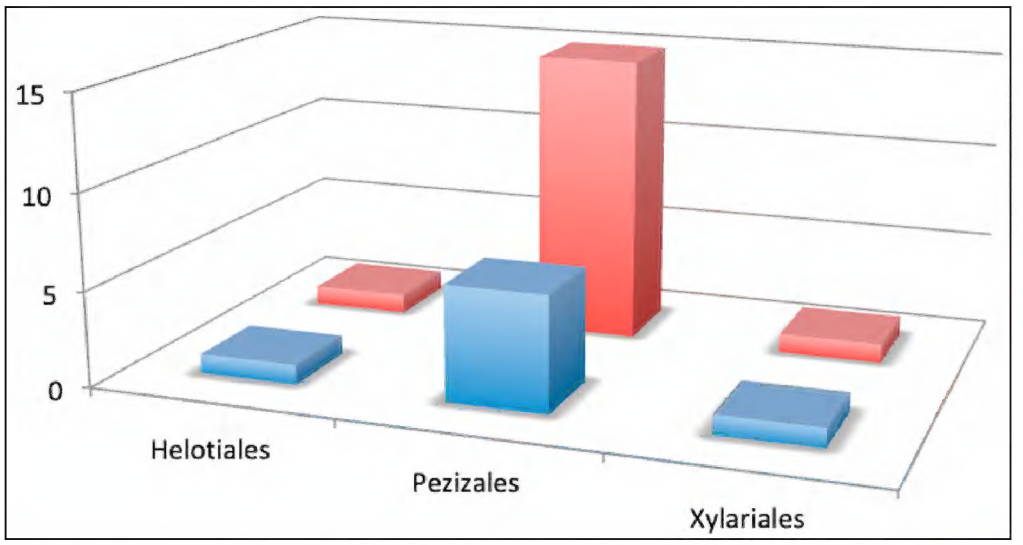

Figure 2. Distribution of macrofungal species and genera per order of Ascomycota: (i) blue columns indicate the number of genera; (ii) red columns indicate the number of species.

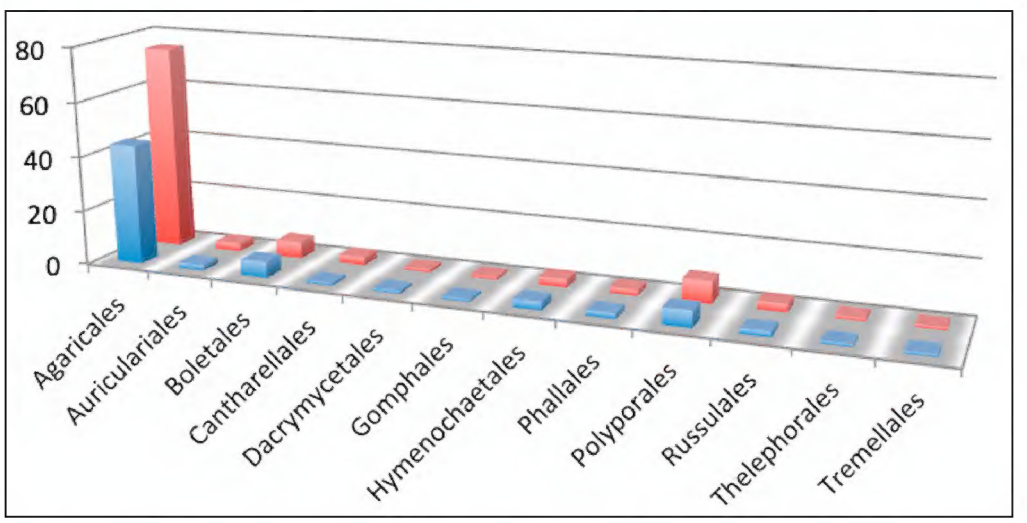

Figure 3. Distribution of macrofungal species and genera per order of Basidiomycota: (i) blue columns indicate the number of genera; (ii) red columns indicate the number of species.

The list of the species with their ecological trophic group is reported in Suppl. material 1: Table S1.

The two main trophic groups were the saprotrophs and ectomycorrhizals, with a total number of 97 and 22 species, respectively.

The saprotrophs were mainly either terrestrial (St) or lignicolous (Sh), which account for 33.6% and 29.6 %, respectively.

The other groups [Pn, Pn(Sh)] collectively represented only 4.8% of the total diversity (Suppl. material 1: Table S1, Fig. 4).

With reference to the Umbrian Checklist of macrofungi (Angelini et al. 2017), 44 species new for the region were found at the Marmore Waterfalls. Thirty-four species included in Red Lists of European countries (http://www.wsl.ch/eccf/) were also found at the site. These species are indicated with the symbols * and ^, respectively, in Suppl. material 1: Table S1.

Fungal community composition varied across the EU-habitat types, as shown in Suppl. material 1: Table S1. Most of the species were sparsely distributed, occurring

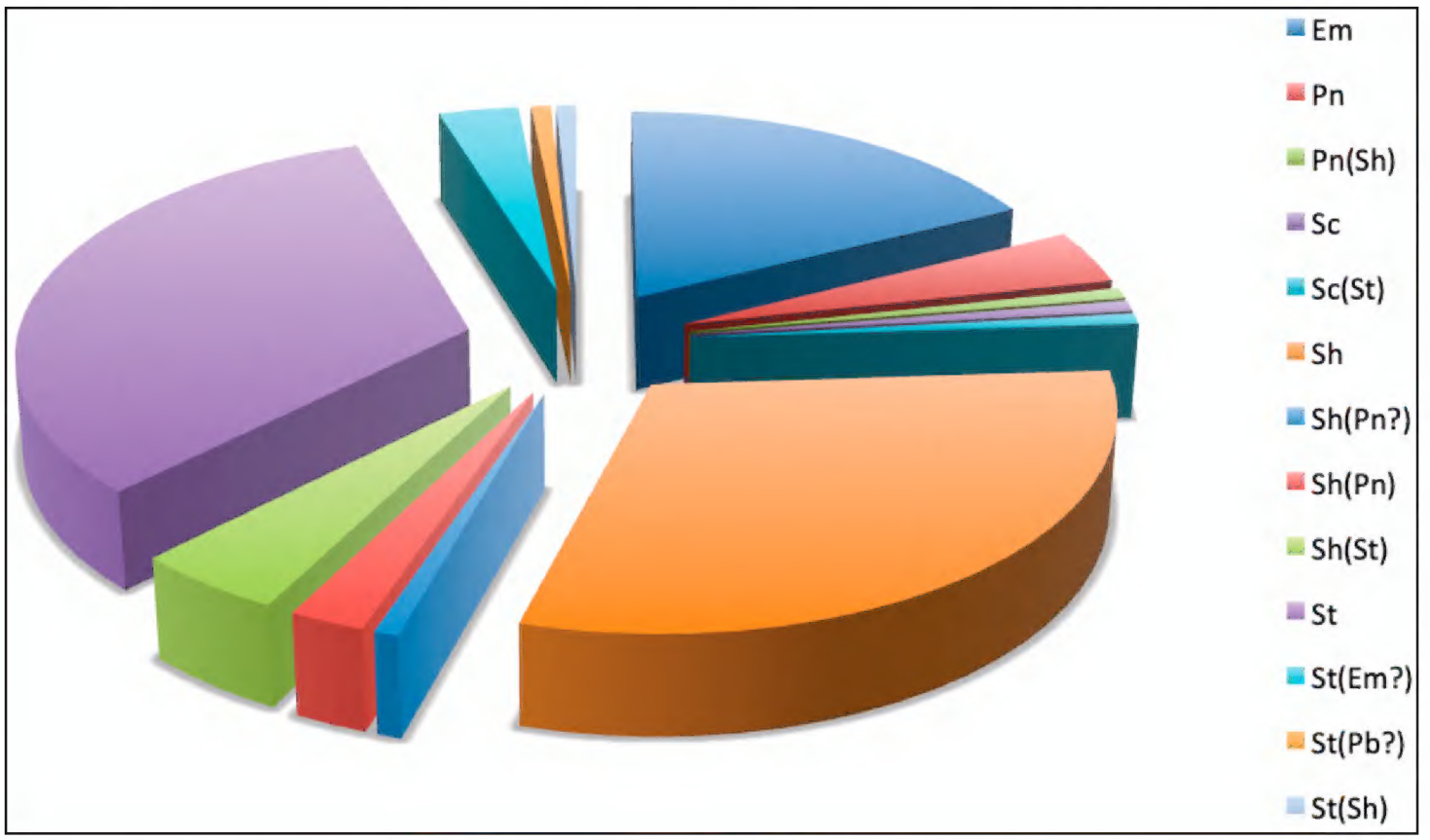

Figure 4. Distribution of macrofungal species per ecological-trophic group (Em, ectomycorrhizal; Pn, necrotrophic parasite; Pn(Sh), necrotrophic parasite or sometimes lignicolous saprotroph; Sc, coprophilous; Sc(St), coprophilous or maybe terrestrial saprotroph; Sh, lignicolous saprotroph; Sh(Pn), lignicolous saprotroph or sometimes necrotrophic parasite; Sh(Pn?), lignicolous saprotroph or maybe necrotrophic parasite; Sh(St), lignicolous saprotroph or sometimes terrestrial saprotroph; St, terrestrial saprotroph; St(Em?), terrestrial saprotroph or maybe ectomycorrhizal; St(Pb?); terrestrial saprotroph or maybe biotrophic parasite).

in few habitat types. More specifically, 108 species were found in only one habitat, 15 were in two types, and 3 in three types.

Regarding the main trophic groups, St species ranged from 22.95% in habitat 9340 to 58.33% in habitat 7220*; Sh species were absent in habitat 6430 and ranged from 22.95% in habitat 9340 to 39% in habitat 92A0. Em species in habitat 9340 (34.42%) were more abundant than saprophic species (Sh or St). Contrarily, in habitats 91E0* and 92A0, Em species were less abundant than Sh/St species. In habitats 6430 and 7220*, Em species were absent (Suppl. material 1: Table S2).

The similarity among EU-habitat types calculated on the basis of presence-absence of fungal species (Jaccard's index) ranged from 0.095 (EU-habitat 9340 vs. EU-habitat 92A0) to 0 (EU-habitat 9340 vs. EU-habitat 7220*) (Suppl. material 1: Table S3). EU-habitats 6340 and 7220* had the most distinctive macrofungal community. *Bolbitius titubans*, *Lacrymaria lacrymabunda* and *Agrocybe vervacti* were restricted to 6340 (Suppl. material 1: Table S1). Some Ascomycota, such as *Helvella corium*, *H. crispa*, *Peziza domiciliana*, *P. queletii*, *Scutellinia scutellata*, and *Tarzetta cupularis* seem to be important biotic components of the priority EU-Habitat 7220* together with some Basidiomycota, such as *Echinoderma calcicola*, *Mycena galopus*, *Phloeomana conopilea*, *P. hiemalis*, *P. speirea*, and *Psathyrella candolleana* (Suppl. material 1: Table S1, Figs S1, S2).

Discussion

This study provided a list of 125 macrofungal species identified at the Marmore Waterfalls over the last ten years (2008–2018) in different Natura 2000 EU-Habitats. Macrofungal communities are structured by host plants/EU-habitats. Similarity was very low among EU-Habitat types, with the exception of 9340 and 92A0 which shared 9 fungal species (*Agaricus moelleri*, *Auricularia mesenterica*, *Calocera cornea*, *Clathrus ruber*, *Schizophyllum commune*, *Lepista nuda*, *L. sordida*, *Tubaria furfuracea*, *T. romagnesiana*).

The dominant tree species of habitats 9340 and 92A0 differed; thus, 9340 showed higher mycorrhizal species richness, while 92A0 had a higher relative number of saprotrophic macrofungi.

Despite the fact that this study is an initial qualitative survey of the macrofungi (based on the presence-absence of species) from the Marmore Waterfalls, it provided a list of 12 fungal species for Habitat 7220*, "Petrifying spring with tufa formation (*Cratoneurion*)", reported here for the first time and never before documented in previous studies in Italy. In general, they are alkalotolerant species (ecological-trophic group: St, terrestrial saprotrophic) and represent, along with the bacteria, the major taxa responsible for decomposing and recycling various organic materials produced by primary producers, the resilient remains of other organisms (bryophytes, algae, protozoans, metazoans, etc.) and dissolved organic compounds (Madigan et al. 2003).

Among the small number of ascomycetes collected during this study, there are five species reported by Wagensommer et al. (2018) as endangered (*Morchella esculenta*, *Scutellinia scutellata*) or "vulnerable" (*Helvella leucomelaena*, *Peziza succosa*, *Saecoscypha coccinea*) in Umbria, because of the threat to their status due to natural or anthropic action.

Of the 34 fungal species included in Red Lists of European countries (http://www.wsl.ch/eccf/) is noteworthy the presence of: (1) *Coprinopsis strossmayeri*, a rare species in Italy (Suppl. material 1: Fig. S3), previously collected only in Piemonte (Vizzini 2001); (2) *Echinoderma calcicola*, a widespread but rare European species, reported only in five other Italian regions (Lombardia, Marche, Piemonte, Toscana, and Veneto) (Onofri et al. 2005). It is considered as "endangered" in the Sweden Red List (Tingstad et al. 2017).

While further study based on fruiting body abundance is needed to provide a measure of the relative importance of a species in EU-habitats, it can be concluded that this ten-year survey has demonstrated that the diversity of the fungi at the Marmore Waterfalls is remarkable, given the high number of species identified in a very small area. The data collected also contribute to draft a naturalistic plan of the Marmore Waterfalls, and provide useful information for monitoring habitats and species of European interest (as required by the Important Plant Areas program and Habitat Directive 92/43/EEC). Important Plant Areas (IPAs) are the most important places in the world for wild plant and fungal diversity, that can be protected and managed as specific sites (Blasi et al. 2009, 2011). The IPA project forms an integral part of a much wider conservation framework, from the global Convention on Biological Diversity framework to regional pan-European and European Union initiatives (Planta Europa

2008, Darbyshir et al. 2017). It gives the possibility of becoming part of conservation actions to countries, habitats and organisms, in some way not considered in the Habitat Directive 92/43/CEE (Perini et al. 2011). Its aim is to identify priority sites using three criteria (threatened species, exceptional botanical richness, and threatened habitats) and to work towards their conservation and management (Anderson 2002, Venturella et al. 2011).

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Supplementary material 1

Supplementary tables and figures

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Data type: species data

Explanation note: **Table S1.** List of macrofungal species collected in the Marmore Waterfalls, Terni, Umbria. **Table S2.** Percentage of relative abundance and number (in parentheses) of fungal species from each ecological group in the Marmore Waterfalls EU- habitats [91E0*, Alluvial forests with *Alnus glutinosa* and *Fraxinus excelsior* (*Alno Padion*, *Alnion incanae*, *Salicion albae*); 92A0, *Salix alba* and *Populus alba* galleries; 6430, Hydrophilous tall herb fringe communities of the plains and the mountains to alpine levels; 7220*, Petrifying springs with tufa formation (*Cratoneurion*); 9340, *Quercus ilex* and *Quercus rotundifolia* forests]. **Table S3.** Similarity of macrofungi communities with respect to EU-habitat [91E0*, Alluvial forests with *Alnus glutinosa* and *Fraxinus excelsior* (*Alno-Padion*, *Alnion incanae*, *Salicion albae*); 92A0, *Salix alba* and *Populus alba* galleries; 6430, Hydrophilous tall herb fringe communities of the plains and of the mountains to alpine levels; 7220*, Petrifying springs with tufa formation (*Cratoneurion*); 9340, *Quercus ilex* and *Quercus rotundifolia* forests], reflecting similarity based on presence/absence data only (Jaccard's index). **Figure S1.** Ascomycota species found in EU Habitat 7220* (Marmore Waterfalls, TR): 1) *Helvella corium* (O. Weberb.) Massee, 2) *H. crispa* (Scop.) Fr., 3) *Peziza domiciliana* Lantieri & Cacialli, 4) *Peziza queletii* Medardi, 5) *Scutellinia scutellata* (L.) Lambotte and 6) *Tarzetta cupularis* (L.) Svrček. **Figure S2.** Basidiomycota species found in EU Habitat 7220* (Marmore Waterfalls, Terni, Umbria): 1) *Echinoderma calcicola* (Knudsen) Bon, 2) *Mycena galopus* (Pers.) P. Kumm, 3) *Parasola conopilus* (Fr.) Örstadius & E. Larss, 4) *Phloeomana hiemalis* (Osbeck) Redhead, 5) *P. speirea* (Fr.) Redhead and 6) *Psathyrella candolleana* (Fr.) Maire. **Figure S3.** *Coprinopsis strossmayeri* (Schulzer) Redhead, Vilgalys & Moncalvo: 1) basidiocarps. Microscopic features (1000×): 2) hyphae with a joint buckle, 3) details of the veil, 4) spores.

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